

with knee OA at baseline and incident OA as a minimum of one point increase in total JSN score in patients with no knee OA at baseline.

Odds ratios were calculated by logistic regression, adjusted for age, sex, body mass index and family effect. The odds ratios were transformed to risk ratios.

**Results:** Of the 208 patients (104 sibling pairs) 193 patients completed the follow-up of two years. The analysis was performed in a total of 154 patients for whom UCTX-II levels at baseline were available and who were either male or postmenopausal female without hormone replacement therapy. The average age was 61 years, and 75% were female. The median (range) UCTX-II level was 243 (44 - 786) ng/mmol creatine for all patients with cut-off points for the middle and high tertile 199 and 306 ng/mmol creatine respectively. In the whole group, 31 patients had an increase in JSN score, 25 with progression of knee OA and 6 with incident knee OA.

77 patients (50%) had knee OA at baseline of whom 25 patients (33%) progressed. The median (range) UCTX-II level was 208 (61 - 700) ng/mmol creatine for patients without progression of OA in the knee and 281 (113-660) for patients with progression of OA in the knee. The median difference was 73 (95% C.I. 11.4 - 134.6) ng/mmol creatine. The adjusted risk ratio for developing radiographic progression in the knee was 5.2 (95% C.I. 3.0 - 6.0) for the middle tertile and 4.5 (95% C.I. 1.7 - 5.9) for the upper tertile of the baseline distribution of UCTX-II. Analysis for patients with progression and incident knee OA combined gave the same results.

**Conclusions:** Patients with an increased UCTX-II level at baseline have an elevated risk to develop radiographic progression of knee OA after two years. However, the range of UCTX-II levels in patients without progression is even wider than the range of UCTX-II in patients with progression. Thus, UCTX-II levels at an individual level cannot predict which patients will show progression in knees with OA in two years.

## P82

### BIOCHEMICAL MARKERS ARE ASSOCIATED WITH RADIOGRAPHIC SUBTYPES OF OSTEOARTHRITIS IN SUBJECT WITH FAMILIAL OA AT MULTIPLE SITES. THE GARP STUDY

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**Purpose:** The extend of OA within each joint is usually assessed by radiographic characteristics and expressed as the Kellgren score. Radiographs are, however, poorly sensitive for monitoring and assessing (the progression of) the disease. Biochemical markers have been identified for bone, cartilage and synovium turnover in humans which may be useful in monitoring more specifically and sensitively the pathophysiological process of OA and may provide a more sensitive prognosis of the patient.

The main objective of this study was to assess the relationship of markers that reflect the process of inflammation, cartilage, bone, and synovium tissue turnover in subjects with radiographic signs of osteoarthritis (ROA) with symptomatic OA at multiple sites (the GARP study) in order to generate hypothesis on the different joint site specific pathophysiological processes that may be reflected by these biochemical markers.

**Methods:** Markers that reflect the process of inflammation, cartilage, bone, and synovium tissue turnover were measured in urine or serum. Urinary CTX-II (UCTX-II) and type IIA procollagen amino terminal propeptide (PIIANP), serum cartilage oligomeric matrix protein (COMP) were measured to represent

cartilage turnover. For bone, serum Osteocalcin (OC), and urinary C-telopeptide of type I collagen (UCTX-I) were assessed, for synovium urinary glucosyl-galactosyl pyridinoline (Glc-Gal-PYD) and for inflammation serum high sensitive C-reactive Protein (HsCRP) were measured. ROA was assessed in the knees, hips, hands, vertebral facet joints and spinal disc degeneration (DD) by the Kellgren and Lawrence score. A proportionate score was made for each joint location based on the number of joints with ROA. Principal component and linear mixed model analysis was used in order to analyze the data.

**Results:** High familial aggregation was observed for S-COMP (70%) and PIIANP (62%), whereas, moderate for UCTX-I (54%) and Glc-Gal-PYD (51%). In our multivariate principal component analyses we found 3 different clusters that may reflect different pathophysiological processes of OA. The first component appeared to be reflected by structural markers of cartilage and bone turnover and associated especially in subjects with hip and hand ROA. The second component was reflected by markers of inflammation and was associated to subjects with knee ROA, high WOMAC scores and BMI. In the third component markers of cartilage turnover clustered together which was associated to ROA at hands, spine and with increasing age.

**Conclusions:** Using a large well-characterized study in which we evaluated most of the available molecular markers for bone, synovial, and cartilage metabolism we were able to observe three components that may reflect different molecular mechanisms; bone turnover, inflammation and synovial degradation. Our data suggested that these components may contribute differently to ROA at different joint site.

## P83

### CONTRIBUTION OF MATRIX METALLOPROTEINASES (MMPs) AND CYSTEINE PROTEASES IN OSTEOARTHRITIS (OA) CARTILAGE DEGRADATION: AN EX-VIVO HUMAN CARTILAGE EXPLANT APPROACH USING THE TYPE II COLLAGEN FRAGMENTS HELIX-II AND CTX-II

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**Purpose:** Background: Cartilage matrix loss including type II collagen is one of the hallmarks of osteoarthritis (OA). Type II collagen degradation can be monitored by measuring the fragments HELIX-II and CTX-II which originate from the triple helical and C-telopeptide regions of the molecule, respectively. Clinical studies have shown that urinary HELIX-II and CTX-II are independently of each other associated with disease progression in OA, suggesting that they may reflect in part different enzymatic pathways of cartilage degradation.

**Objective:** To investigate the relative contribution of endogenous MMPs and cysteine proteases- the two major classes of enzyme believed to play a role in cartilage degradation in OA- on the release of HELIX-II and CTX-II from unstimulated human cartilage explants *in vitro*

**Methods:** Full-depth cartilage from patients with OA undergoing knee replacement were immediately deep frozen at -70°C to preserve endogenous enzyme activity until assay. Sections were then cut from frozen biopsies (200 µm thick), washed twice with PBS and then incubated for 24, 48 or 96 hrs, at 37°C and gentle agitation in buffers, which allowed activity of cysteine proteases (pH 5.5+ cysteine) or of MMPs (pH 7.5+ APMA, an activator of latent MMPs). In the same experiments, the activity of cysteine proteases was inhibited by the non-selective inhibitor E-64 and that of MMPs by GM6001. After incubation, the supernatant was isolated, centrifuged (10 000 rpm), and the supernatant was kept

at -70°C until assay for Helix-II and CTX-II by ELISA (Syncart™ and Cartilaps™, respectively)

**Results:** When cartilage sections were incubated in a buffer favorable for cysteine-proteases, an increase of the release of HELIX-II was observed after 96 hours of incubation. This increase was completely abolished by the addition of E64 in culture medium. In presence of an MMP-buffer and APMA, HELIX-II also time-dependently increased and addition of GM6001 only partially blunted this effect. A time-dependent increase of CTX-II was also observed when cartilage explants were incubated in cysteine-protease or MMP buffers. This increase was completely suppressed by GM6001 and only partially with E-64.

**Conclusions:** Using a non-stimulated ex-vivo human cartilage explant model, which is responding to the endogenous proteinases within cartilage *in vivo*, we found that both cysteine proteases and MMPs are involved in the release of HELIX-II and CTX-II, although the relative contribution of these two enzymatic pathways may differ for the two biochemical markers. These findings should be useful for a better understanding of the pathophysiological pathways of cartilage degradation in OA and for interpretation of clinical findings obtained with HELIX-II and CTX-II

## P84

### METHODS FOR QUANTITATIVE HISTOPATHOLOGY IN OSTEOARTHRITIS RESEARCH

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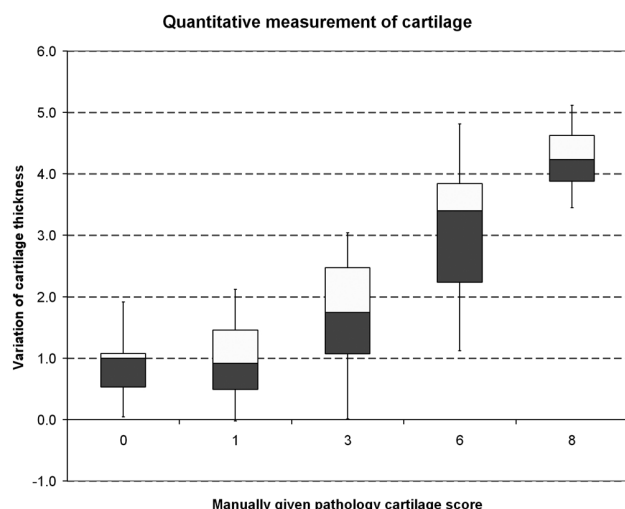
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**Purpose:** The growing interest in automation and quantitative histopathological end-points, based on image analysis, is motivated by a number of current limitations: 1) Replacing manual scoring methods with quantitative end-points would potentially increase sensitivity of measurements and reduce/eliminate inter- and intra grader variability, 2) Quantitative end-points will allow for the use of stronger parametric tests. 3) Reduction of manual labor. Validated methods are currently not available for the research community.

To develop robust and generally applicable automated image analysis algorithms for quantifying cartilage destruction, cellularity, and bone density based on histological sections, capable of reflecting grades obtained using widely used grading methods.

**Methods:** 197 H&E stained histological knee sections were obtained from 50 STR/1N mice sacrificed at the age of 12 weeks. Each section was graded manually according to the modified Mankin score with respect to cartilage, bone, and cellularity.



Based on a previously developed image segmentation method, image analysis algorithms were developed for quantifying: 1) Irregularity of cartilage surface based on variation in cartilage thickness, 2) Bone sclerosis, and 3) Cellularity measured as area fraction of chondrocytes. Each of these quantitative end-points were correlated against the respective manual grading scores of cartilage destruction, bone density, and cellularity. The algorithms were implemented based on Imaging Utilities SDK and the Visiopharm Integrator System.

**Results:** Significant and high correlations between quantitative and semi-quantitative manual grades were found for all three aspects of joint degradation:  $r(\text{cartilage}) = 0.78$ ,  $r(\text{bone}) = 0.67$ ,  $r(\text{cell}) = -0.43$ , with significance levels  $p < 0.001$  for all quantitative end-points.

Box plots of the results for the three end-points, correlated with pathology score, are illustrated in the figures.

